



UNIVERSITI PUTRA MALAYSIA

**PURIFICATION, CHARACTERIZATION AND MOLECULAR STUDIES
OF FRUCTOSE-6-PHOSPHATE PHOSPHOKETOLASE (F6PPK)
FROM BIFIDOBACTERIA**

KHALID GHAZI DAIFALLA FANDI

FSMB 2001 14

**PURIFICATION, CHARACTERIZATION AND MOLECULAR
STUDIES OF FRUCTOSE-6-PHOSPHATE PHOSPHOKETOLASE
(F6PPK) FROM BIFIDOBACTERIA**

KHALID GHAZI DAIFALLA FANDI

DOCTOR OF PHILOSOPHY

UNIVERSITI PUTRA MALAYSIA

2001



**PURIFICATION, CHARACTERIZATION AND MOLECULAR STUDIES OF
FRUCTOSE-6-PHOSPHATE PHOSPHOKETOLASE (F6PPK) FROM
BIFIDOBACTERIA**

By

KHALID GHAZI DAIFALLA FANDI

**Thesis Submitted in Fulfilment of the Requirement for the Degree of
Doctor of Philosophy in the Faculty of Food Science and Biotechnology
Universiti Putra Malaysia**

June 2001



DEDICATION

This dissertation is dedicated to my beloved parents, Ghazi Al-Zoubi and Fatemah Al-Zoubi, for their constant love and inspiration. To my wife Bayan and my cute son Omar, for their love and patience in a long-distance relationship.

Abstract of the thesis presented to the Senate of Universiti Putra Malaysia in
fulfilment of the requirement for the degree of Doctor of Philosophy

**PURIFICATION, CHARACTERIZATION AND MOLECULAR STUDIES OF
FRUCTOSE-6-PHOSPHATE PHOSPHOKETOLASE (F6PPK) FROM
BIFIDOBACTERIA**

By

KHALID GHAZI DAIFALLA FANDI

June 2001

Chairman: Prof. Dr. Hasanah Mohd. Ghazali

Faculty: Food Science and Biotechnology

Fructose-6-phosphate phosphoketolase (F6PPK; EC 4.1.2.22) is the key enzyme in the fructose-6-phosphate shunt pathway of glucose metabolism which is apparently restricted to bifidobacteria. Despite the biological importance of this bacterial group and the heterogeneity of the enzyme from different species, F6PPK in itself has not been characterized in detail with respect to size, subunit number, steady kinetics and N-terminal sequence. F6PPK was extracted and characterized for the first time from *Bifidobacterium asteroides* (isolated from the intestine of honeybees; ATTC 25909). The enzyme was purified to homogeneity using acetone fractionation at 40-70% saturation followed by fast protein liquid chromatography (FPLC) on Mono-Q anion exchange and Superose 12 gel filtration columns. The intact enzyme has a relative molecular mass of 110 ± 5 kDa as estimated by gel filtration chromatography (Sephadex G-200), and a single band was obtained on nondenaturing

PAGE. It was then shown to be that of F6PPK following elution from preparative polyacrylamid gel. Sodium dodecyl sulphate (SDS)-PAGE under nonreducing conditions revealed the presence of a single polypeptide of 110 ± 2 kDa. SDS-PAGE of F6PPK reacted with 2-mecaptoethanol revealed the presence of two polypeptides of 59 ± 1 and 53 ± 0.5 kDa, indicating a dimeric structure ($\alpha_1 \beta_1$) with disulfide-linked subunits. The NH_2 -terminal amino acid of the α chain was found to be methionine. The enzyme was stable at pH 4.5-8.0 with an optimum activity at pH 6.0. The enzyme was stable below 42°C and the optimum temperature was 30°C . The apparent K_m value of the enzyme for fructose-6-phosphate was 14.1 mM. The purified enzyme has no apparent requirement for thiamine pyrophosphate as cofactors. The enzyme was inactivated by Hg^{2+} and recovered after addition of dithiothretol, indicating that sulfhydryl group was probably involved in the enzyme activity. The features of *B. asteroides* F6PPK showed marked differences from those previously reported from animal and human strains.

F6PPK from *Bifidobacterium longum* (probiotic grade; BB536) was also purified to electrophoretic homogeneity using the same purification steps above. The purified enzyme had a molecular mass of about 300 kDa as determined by gel filtration on Superose 12. F6PPK migrated as a single electrophoretic band in non-denaturing polyacrylamide gel electrophoresis (PAGE). It is probably a tetramer containing two different subunits with molecular masses of about 93 ± 1 kDa and 59 ± 0.5 kDa, as determined by SDS-PAGE. The N-terminal amino acid sequences of the subunits were determined, and no significant similarity was found between the

deduced amino acid sequences and those in the databases of EMBL and SWISS-PORT, indicating that we may be reporting for the first time the partial sequence of F6PPK from two type strains of *Bifidobacterium* species. However, the Mr 59 000 subunit of *B. asteroides* F6PPK showed a significant similarity (70%) with the corresponding subunit from *B. longum* species.

Oligonucleotide probes which were designed based on the deduced N-terminal amino acids sequences were unable to detect the presence of F6PPK gene using dot blot and Southern blot of the total genomic DNA from different species of bifidobacteria and other bacterial strains. In addition, the genomic library of *B. asteroides* was constructed in *Bam*HI-digested pUC19 by using about 2 to 6-kb DNA fragments obtained by partial digestion of the total genomic DNA with *Bam*HI. The transformed cells efficiency of *E. coli* XL1- blue carrying plasmids with genomic inserts was 1.1×10^4 cfu ml⁻¹, and this library may be a useful tool for fishing the gene encoding for F6PPK.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk ijazah Doktor Falsafah

**PENULINAN, PENCIRIAN DAN KAJIAN MOLEKUL KE ATAS
FRUKTOSA-6-FOSFAT FOSFOKETOLASE (F6PPK) DARI
BIFIDOBACTERIA**

Oleh

KHALID GHAZI DAIFALLA FANDI

Jun 2001

Pengerusi: Prof. Dr. Hasanah Mohd. Gazali

Fakulti: Sains Makanan dan Bioteknologi

Fruktosa-6-fosfat fosfoketolase (F6PPK; EC 4.1.2.22) merupakan enzim utama dalam lintasan penyimpangan fruktosa-6-fosfat dalam metabolisme glukosa yang terbatas kepada bifidobacteria. Walaupun kumpulan bakteria ini mempunyai kepentingan biologi yang tersendiri dan mempunyai sifat F6PPK yang berbeza dari spesis ke spesis, enzim ini masih belum pernah dicirikan secara terperinci dari segi saiz, bilangan subunit, kinetik tetap dan jujukan terminal-N. F6PPK daripada *Bifidobacterium asteroides* (dipencil dari usus lebah madu; ATCC 25909) telah diekstrak dan dicirikan buat pertama kalinya. Enzim ini telah dituliskan sehingga homogen dengan menggunakan pemeringkatan aseton pada tahap ketepuan 40-70% diikuti dengan kromatografi cecair protein pantas (FPLC) menggunakan turus penukaran anion Mono-Q dan penurasan gel Superose 12. Enzim yang sempurna

mempunyai jisim molekul relatif sebanyak 110 ± 2 kDa menggunakan kromatografi penurasan gel (Sephadex G-200). Jalur tunggal telah diperolehi melalui PAGE ternyahasli, dan disahkan enzim F6PPK setelah protein dielusi daripada gel poliakralamid penyediaan. Dibawah keadaan ternyahasli, natrium dodesil sulfat (SDS)-PAGE telah menunjukkan kehadiran satu polipeptida berjisim 110 ± 5 kDa. SDS-PAGE ke atas F6PPK yang telah ditindakbalas dengan 2-merkaptioetanol telah menunjukkan kehadiran dua polipeptida yang masing-masing berjisim 59 ± 1 dan 53 ± 0.5 kDa. Pencerapan ini menunjukkan satu struktur dimerik ($\alpha_1 \beta_1$) dengan subunit-subunit yang diikat dengan ikatan disulfida. Asid amino terawal di hujung NH_2 telah dikenalpasti sebagai metionin. Enzim telah didapati stabil pada pH 4.5-8.0 dengan aktiviti optimum pada pH 6.0. Enzim ini juga stabil pada suhu di bawah 42°C dan suhu optimumnya ialah 30°C . Nilai K_m nyata bagi enzim ini untuk fruktosa-6-fosfat ialah 14.1 mM. Enzim yang telah ditulinkan tidak memerlukan tiamin pirofosfat sebagai ko-faktor. Enzim ini telah ternyahaktif dalam kehadiran Hg^{2+} dan diaktifkan semula selepas penambahan ditiotretol. Pencerapan ini menunjukkan bahawa kumpulan sulfidril berkemungkinan terlibat dalam aktiviti enzim. Sifat-sifat F6PPK dari *B. asteroides* telah menunjukkan perbezaan yang ketara apabila dibandingkan dengan ciri enzim F6PPK yang diasingkan daripada haiwan dan manusia.

F6PPK daripada *Bifidobacterium longum* (gred probiotik; BB536) juga telah ditulinkan sehingga homogen secara elektroforetik dengan menggunakan langkah-langkah penulinan seperti di atas. Jisim molekul enzim ini dianggarkan sekitar

300 kDa, melalui penurasan gel dengan Superose 12. F6PPK telah bergerak sebagai satu jalur yang tunggal dalam PAGE tak-ternyahasli. Ia bermungkinan merupakan satu tetramer yang terdiri dari dua subunit yang berlainan, masing-masing berjisim sekitar 93 ± 1 dan 59 ± 0.5 kDa, seperti yang ditentukan melalui SDS-PAGE. Jujukan asid amino dari hujung N bagi subunit-subunit enzim telah ditentukan dan didapati tiada persamaan nyata diantara jujukan asid amino ini dengan jujukan asid amino lain dalam databes EMBL dan SWISS-PORT. Ini menunjukkan bahawa kami berkemungkinan melaporkan buat kali pertama jujukan sebahagian F6PPK daripada dua jenis spesis *Bifidobacterium*. Walau bagaimanapun, subunit M_r 59 000 daripada F6PPK dari *B. asteroides* menunjukkan kesamaan yang nyata (70%) dengan subunit berpadan daripada spesis *B. longum*.

Prob oligonukleotida yang telah direka berdasarkan jujukan asid amino hujung N didapati tidak mampu mengesan gen bagi F6PPK apabila pemblotan bintik dan pemblotan Southern dilakukan ke atas keseluruhan DNA genomik dari pelbagai spesis bifidobacteria dan lain-lain jenis bakteria. Sebagai tambahan, sumber data genomik bagi *B. asteroides* telah dibina dalam pUC19 terhadap-*Bam*HI dengan menggunakan sebanyak 2 hingga 6-kb pecahan DNA yang diperolehi dari penghadaman separa ke atas DNA genomik dengan enzim pembatas *Bam*HI. Kecekapan sel-sel *E. coli* XL-1 biru yang telah ditransfomasi untuk membawa plasmid yang mempunyai selitan genomik adalah 1.1×10^4 cfu ml⁻¹. Sumber data ini boleh digunakan untuk mencari gen yang mengkod F6PPK.

ACKNOWLEDGEMENTS

First and foremost, I would like to express my deepest thanks and appreciation to my advisor Prof. Dr. Hasanah M. Ghazali of the Department of Biotechnology, Faculty of Food Science and Biotechnology for the opportunity to work in her lab, for her guidance throughout the course of my study and for the confidence she has in me. Thanks are due to her also for her interesting project, from which I gained valuable research experience and scientific knowledge. I would like also to thank, Dr. Raha Abdul Rahim, Dr. Mohd. Yazid and Dr. Takashi Tamura, who were gracious to serve on my thesis committee, for their helpful comments, suggestions on my project and for reviewing dissertation manuscripts.

Furthermore, I would like to thank my friends and colleagues during my graduate study for their help and for their insight and humor. The friendship that I have had with them will always be cherished.

Acknowledgement is also due to the Government of Malaysia and Universiti Putra Malaysia for granting me a research assistantship, to carry out the PhD program. I also wish to thank JICA, Japan, for their contributions of some equipment especially the FPLC, without which this study is yet to be completed.

Finally, to my family.... my wife Bayan and my son Omar who was born during the course of my study, and who help me, with his smiles, understand the meaning of life. I would not be able to write this today if it wasn't for all of you. How can I possibly thank you for all the love, support, understanding, and encouragement that you have given to me over the years. Thank you for not giving up on me and for putting up with me at my worst times. Though physically many miles away, my brothers and sisters have been very caring and morally supportive of my work. Thank you all for all your prayers. I am grateful to my parents-in-law who are always supportive with their love and understanding and taking care of my wife and son when I am far from them. To my father and mother, there are no words kind enough to describe you, and no words adequate to thank you. I only wish that everyone could have parents like you. Through your unending love and total support I have been able to achieve my dreams, this one is for you.



I certify that an Examination Committee met on the 12th of June 2001 to conduct the final examination of Khalid Ghazi Daifalla Fandi on his Doctor of Philosophy thesis entitled "Purification, Characterization and Molecular Studies of Fructose-6-phosphate phosphoketolase (F6PPK) from Bifidobacteria" in accordance with Universiti Pertanian Malaysia (Higher Degree) act 1980 and Universiti Pertanian Malaysia (Higher Degree) Regulation 1981. The committee recommends that the candidate be awarded the relevant degree. Members of the Examination committee are as follows:

Jamilah Bakar, Ph.D.
Professor Madya
Faculty of Food Science and Biotechnology
Universiti Putra Malaysia
(Chairperson)

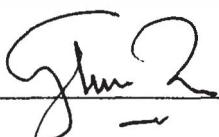
Hasanah Mohd. Ghazali, Ph.D.
Professor
Faculty of Food Science and Biotechnology
Universiti Putra Malaysia
(Member)

Mohd Yazid Abdul Manap, Ph.D.
Professor Madya
Faculty of Food Science and Biotechnology
Universiti Putra Malaysia
(Member)

Raha Abdul Rahim, Ph.D.
Faculty of Food Science and Biotechnology
Universiti Putra Malaysia
(Member)

Takashi Tamura, Ph.D.
Department of Bioresources Chemistry
Faculty of Agriculture
Okayama University, Japan
(Member)

Kenji Inagaki, Ph.D.
Professor
Faculty of Agriculture
Okayama University, Japan
(Independent Examiner)



MOHD. GHAZALI MOHAYIDIN, Ph.D.,
Professor/ Deputy Dean of Graduate School,
Universiti Putra Malaysia
Date: _____

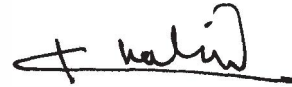
This thesis submitted to the Senate of Universiti Putra Malaysia has been accepted as fulfilment of the requirement for the degree of Doctor of Philosophy.



AINI IDERIS, Ph.D.
Professor,
Dean of Graduate School,
Universiti Putra Malaysia

Date:

I hereby declare that the thesis is based on my original work for quotations and citations, which have been duly acknowledged. I also declare that it has not been previously or currently submitted for any other degree at UPM or other institutions.



KHALID GHAZI DAIFALLA FANDI

13/6/2001

TABLE OF CONTENTS

	Page
DEDICATION	2
ABSTRACT	3
ABSTRAK	6
ACKNOWLEDGEMENTS	9
APPROVAL SHEETS	11
DECLARATION FORM	13
LIST OF TABLES	18
LIST OF FIGURES	19
LIST OF PLATES	22
LIST OF ABBREVIATIONS	23

CHAPTER

1	INTRODUCTION	25
2	LITERATURE REVIEW	32
	Background	32
	Gastrointestinal Ecology	33
	Bifidobacteria	36
	General Characteristics.....	36
	Taxonomy.....	38
	Identifications Methods and Molecular Approaches of Bifidobacteria	39
	Biochemistry and Metabolic Pathway	45
	Hexose Metabolism	45
	Fructose-6-phosphate Shunt	48
	Fructose-6-phosphate phosphoketolase	50
	Development of Bifidobacteria in the Human Colon	57
	<i>Bifidobacterium</i> : A Natural Inhabitant of the Intestine ...	60
	Surface Properties	61
	Stomach and Intestinal Transit	62
	Bifidobacteria as Probiotics	63
	Bifidobacteria and Enteric Microflora Modulation	65
	Health Effects of Bifidobacteria	68
	Immunomodulating Effects	68
	Reduction of Risk Cancer	70
	Antimicrobial Activity	72
	Improve Gastrointestinal Transit	73
	Other Health Effects	74
	Bifidobacteria as Dietary Adjuncts	75
	Application of Bifidobacteria in Food Industry	77
	<i>In Vivo</i> Consumption of Probiotic and Prebiotic Products.....	80

3	EXTRACTION, PURIFICATION AND PROPERTIES OF FRUCTOSE-6-PHOSPHATE PHOSPHOKETOLASE FROM <i>BIFIDOBACTERIUM ASTEROIDES</i>.....	83
	Introduction.....	83
	Materials and Methods.....	86
	Materials.....	86
	Strain, Cultivation and Harvest of Cells.....	86
	Preparation of Cell Extracts.....	87
	Preparation of Extract for Characterization	88
	Assay of Fructose-6-phosphate phosphoketolase Activity.....	89
	Estimation of Protein Concentration.....	90
	Gel Electrophoresis.....	90
	Purification of F6PPK.....	92
	Acetone precipitation.....	92
	Mono Q anion-exchange chromatography.....	92
	Superose 12 gel filtration chromatography.....	93
	Determination of F6PPK Purity	94
	Gel Staining and Drying.....	95
	Characterization of F6PPK.....	95
	Results and Discussion.....	99
	Cell Cultivation and Harvest.....	99
	Partial Purification of F6PPK.....	100
	Purification of F6PPK from <i>B. asteroides</i>	104
	Detection of F6PPK After Electrophoresis.....	110
	Properties of F6PPK.....	113
	Summary.....	127
 4	 N-TERMINAL AMINO ACID SEQUENCE OF PURIFIED FRUCTOSE-6-PHOSPHATE PHOSPHOKETOLASE FROM <i>B. LONGUM</i> AND <i>B. ASTEROIDES</i>	 129
	Introduction.....	129
	Materials and Methods.....	131
	Materials.....	131
	Bacterial Strains and Their Cultivation.....	132
	Preparation of Crude Extract.....	132
	Analytical Procedures.....	133
	Purification of F6PPK from <i>B. longum</i>	133
	Determination of the Molecular Masses and Purity	135
	Protein Electrophoresis for Sequencing.....	136
	Determination of NH ₂ -terminal Sequence.....	137
	Results and Discussion.....	139
	Purification of F6PPK from <i>B. longum</i>	139
	Molecular Mass Determination.....	143
	N-terminal Amino Acid Sequence Analysis.....	148
	Summary.....	151

5	DETECTION OF BIFIDOBACTERIA USING OLIGONUCLEOTIDE PROBE DESIGNED FROM N-TERMINAL AMINO ACIDS SEQUENCE OF F6PPK.....	152
	Introduction.....	152
	Materials and Methods.....	154
	Materials.....	154
	Bacterial Strains and Growth Conditions.....	155
	Chromosomal DNA Preparation.....	155
	DNA Blot and Hybridization Analysis.....	158
	Preparation of DIG-labeled Probe.....	160
	Southern and Dot Blot Hybridization	160
	Detection Procedure.....	162
	Results and Discussion.....	163
	Isolation of Total Genomic DNA.....	163
	Probe Design.....	163
	Southern and Dot Blot Hybridizations.....	166
	Summary.....	171
 6	 CONSTRUCTION OF A GENOMIC LIBRARY FOR <i>BIFIDOBACTERIUM ASTEROIDES</i>.....	 172
	Introduction.....	172
	Material and Methods.....	174
	Materials.....	174
	Isolation of Genomic DNA	175
	Quantification of Genomic DNA.....	176
	DNA Manipulation.....	178
	Preparation of Competent Cells.....	180
	Transformation and Storage of the Genomic Library.....	180
	Miniprep Plasmid Isolation.....	181
	Results and Discussion.....	183
	Isolation and Partial Digestion of Genomic DNA.....	183
	Dephosphorylation of Plasmid DNA and Ligation with Genomic DNA.....	186
	Transformation.....	188
	Isolation of Insert Plasmid.....	182
	Summary.....	185

7	SUMMARY, CONCLUSION AND RECOMMENDATIONS	196
	Summary	196
	Conclusion and Recommendations	199
	BIBLIOGRAPHY	201
	APPENDICES	225
	BIOGRAPHICAL SKETCH	238

LIST OF TABLES

Table		Page
1	The Electrophoretic Mobility of Fructose-6-phosphate phosphoketolase in <i>Bifidobacteria</i>	54
2	Comparison of F6PPK Properties From Different <i>Bifidobacterium</i> Strains	55
3	Summary of Partial Purification of F6PPK From <i>B. asteroides</i>	101
4	Purification of Fructose-6-phosphate phosphoketolase (F6PPK) From <i>B. asteroides</i>	105
5	Effect of Different Inhibitors and Cations on <i>B. asteroides</i> F6PPK	120
6	The Effect of Thiamine-PP and Mg^{++} on F6PPK Activity	124
7	Summary of Purification of F6PPK From <i>B. longum</i>	140
8	Comparison of F6PPK Properties From Different <i>Bifidobacterium</i> Strains	142
9	Designation and Origin of Bacterial Strains used in This Study	156
10	Oligonucleotide Probes Used in Hybridization Assays	161

LIST OF FIGURES

Figure		Page
1	Pathway of Glucose Metabolism and Erythritol Formation in <i>Leuconostoc oenos</i>	46
2	Formation of Acetate and Lactate from Glucose by Bifidum Pathway and by the Homofermentative Pathway	49
3	Difference in Species of Bacteria in Human Feces of Different Ages	59
4	Sephadex G-200 Column Chromatography	102
5	Electrophoresis of Cell Extract from <i>B. asteroides</i> F6PPK at Each Purification Step Through a 7.5% Polyacrylamid Gel Electrophoresis.	103
6	Elution Profile of F6PPK Activity of <i>B. asteroides</i> From Mono Q column	106
7	Superose 12 chromatography of F6PPK From <i>B. asteroides</i>	108
8	Electrophoresis of F6PPK From <i>B. asteroides</i> at Various Steps of Purification	109
9	Electrophoresis of Cell Extracts Through a 7.5% Nondenaturing Polycarylamide Gel	111
10	SDS-PAGE of Purified F6PPK from <i>B. asteroides</i>	112
11	Effect of pH on F6PPK Activity	114
12	Effect of pH on F6PPK Stability	115
13	Effect of Temperature on F6PPK Activity	116
14	Effect of Thermostability on F6PPK Activity	117
15	The Effect of Hg ⁺² and Dithiothreitol on F6PPK Activity	119
16	Michaelis-Menten Kinetic Profiles of F6PPK From <i>B. asteroides</i>	122

17	Estimation of the Relative Molecular Mass of the Native F6PPK by Gel Filtration on Sephadex G-200 Column	126
18	SDS-PAGE on PVDF Membrane	138
19	Elution Profile of F6PPK Activity of <i>B. longum</i> From Mono Q Column	141
20	Superose 12 chromatography of F6PPK From <i>B. longum</i>	144
21	Gradient PAGE of F6PPK Fractions from <i>B. longum</i>	145
22	Determination of the Apparent Molecular Mass of the Native F6PPK from <i>B. longum</i> by Gel Filtration on Superose 12 Column	146
23	SDS Polyacrylamide Electrophotograms of Fractions Obtained During the Purification of F6PPK From <i>B. longum</i>	147
24	Alignment of the N-terminal Amino Acid Sequences (14 to 23 amino acids) of the F6PPK Subunits Purified From Three Strains of <i>Bifidobacterium</i>	150
25	Total Chromosomal DNA Isolated from Different Bacterial Species	164
26	Southern Hybridization Analysis of the Genomic DNA Digested with <i>Bam</i> HI and Blotted to Nylon Membrane	165
27	Southern Hybridization Analysis Correspondences to the Gel in Figure 2 Using F6PPK Probes	167
28	Dot blot Hybridization Between Total DNA and F6PPK probes BA59 (a), and BL93-2 (b)	169
29	Restriction Map of the Plasmid pUC19. The lower part of the figure details the polylinker cloning sites	171
30	Genomic DNA Isolation and Small Scale Partial Digestion of <i>B. asteroides</i> DNA	184
31	Genomic DNA and Plasmid pUC19 Digestion with <i>Bam</i> HI	187
32	Isolation of Transformed Plasmids from Genomic Library of <i>B. asteroides</i>	193

33	Typical Standard Curve for the Acetyl-Phosphate Assay for Fructose-6-phosphate	233
34	Typical Standard Curves for Protein Assay for BSA and Bio-Rad Solution	234
35	Gel-Filtration Chromatogram of Standard Proteins and F6PPK Eluted on Sephadex G-200 Column	236
36	Gel-Filtration Chromatogram of Standard Proteins (MW-GF-1000) Eluted on Superose 12 Column Using FPLC.	237

LIST OF PLATES

Plates		Page
1	Transformation of host cell with uncut pUC19	190
2	Transformation of <i>E. coli</i> XL1-Blue with recombinant molecules (inserts from <i>B. asteroides</i>)	190
3	Transformation of <i>E. coli</i> DH5 α with recombinant molecules (inserts from <i>B. asteroides</i>)	191

LIST OF ABBREVIATIONS

Abbreviated	Full Form
Ab	Absorbance
AP	Alkaline phosphatase
APS	Ammonium Persulphate
ATCC	American Type Culture Collection
BSA	Bovine serum albumin
°C	Degree Celsius
CFU	Colony forming unit
CIAP	Calf intestinal alkaline phosphatase
Da	Dalton
DNA	Deoxyribonucleic acid
dATP	Deoxy adenine triphosphate
dCTP	Deoxy cytidine triphosphate
dGTP	Deoxy guanine triphosphate
dTTP	Deoxy thymidine triphosphate
DTT	Dithiothreitol
EDTA	Ethylene diamine tetraacetic acid
<i>E.coli</i>	<i>Escherichia coli</i>
EtBr	Ethidium bromide
F6P	Fructose-6-phosphate
F6PPK	Fructose-6-phosphate phosphoketolase
FPLC	Fast protein liquid chromatography
g	gravity
gm	Gram
h	hour
IPTG	Isopropylthio- β -D-galactoside
Kb	Kilobase
kDa	KiloDalton
K_m	Michaelis constant
LB	Luria broth
L.M.P	Low melting piont
mg	Milligram
μ g	Microgram
l	litre
ml	Millilitre
mol	mole
mm	millimeter
μ M	Micromolar
M	Molar (mol/l)
Met	methionine
MnCl ₂	Manganese chloride
MWCO	Molecular weight cut off